CHROMSYMP. 2449

Factors affecting the separation of inorganic metal cations by capillary electrophoresis

Andrea Weston and Phyllis R. Brown*

Department of Chemistry, University of Rhode Island, Kingston, RI 02881 (USA)

Petr Jandik, William R. Jones and Allan L. Heckenberg

Ion Analysis Department, Waters Chromatography Division of Millipore, 34 Maple St., Milford, MA 01757 (USA)

ABSTRACT

Various alkali metals, alkaline earth metals, transition metals and lanthanides were separated by capillary electrophoresis, and factors influencing the separations were studied. The reproducible separation of fifteen metal cations was completed in 8 min. The detection system showed a linear relationship between peak area and analyte concentration. To permit the use of indirect photometric detection and to ensure symmetrical peak shapes, a highly UV-absorbing amine having an electrophoretic mobility similar to those of the analyte cations was chosen as the major component of the electrolyte. Complexing compounds were added to the electrolyte to maximize selectively the differences in the apparent mobilities of the cations and enhance the separations.

INTRODUCTION

In recent years, capillary electrophoresis (CE) has been used mainly for the separation of biological macromolecules [1–3]. Fewer papers have been published relating to inorganic compounds. Of these papers, the majority report separations of inorganic anions. The separations of anions by CE have been shown to be highly sensitive (nanomolar limits of detection) and highly efficient (20 000–1 000 000 theoretical plates) [4–7]. They offer a selectivity which is complementary to existing techniques such as ion chromatography. Although the first published application of CE for inorganic cations was reported in 1967 by Hjerten [8], few applications of CE for inorganic cations have since been published [9–11].

A recent paper on cation separations was published by Foret *et al.* [12]. In this method, indirect UV detection was utilized for the separation of a group of lanthanide cations. Complexing agents were added to the electrolyte to effect a highly efficient separation in under 5 min. A different complexing agent was employed by Swaile and Sepaniak [13] for the laser-based fluorescence detection of three common metal ions. The effect of pH and complexing agent concentration on the separation of the three metal cations was described.

We report here several successful separations of Group IA, Group IIA, transition metal and lanthanide cation mixtures by CE, with indirect UV detection, and some method development rules unique to the separation of inorganic cations.

EXPERIMENTAL

A Waters (Milford, MA, USA) Quanta 4000 capillary electrophoresis system, equipped with a positive power supply, was used. Fused-silica capillaries, 75 μm I.D. and 52 cm from the point of sample introduction to the detector window, were obtained from Waters (AccuSep capillaries). Indirect UV detection was achieved with the use of a zinc lamp and a 214-nm optical filter. The samples were introduced into the capillary by 20- or 30-second hydrostatic injections from a height of 10 cm.

Standard 2-ml polyethylene sample vials (Sun Brokers, Wilmington, NC, USA) were used as containers for the carrier electrolyte and for all the standards and samples. A Waters 860 Data Station and Waters SIM interface were used to record and evaluate the electropherograms, the subsequent statistical processing being performed using Cricket-Graph (Cricket Software, Malvern, PA, USA) with a Macintosh SE personal computer (Apple Computers, Cupertino, CA, USA).

All solutions, electrolytes and standards were prepared using 18-MΩ water generated by a Milli-Q laboratory water purification system (Millipore, Bedford, MA, USA). The transition metal standards were prepared by the dilution of standards obtained from Sigma (St. Louis, MO, USA), and the alkali and alkaline earth metal standards were prepared from salts obtained from Aldrich Chemical (Milwaukee, WI, USA), as was the analytical reagent-grade α-hydroxyisobutyric acid (HIBA). The UV background-providing component of the electrolyte, UVCat 1, was obtained from Waters.

RESULTS AND DISCUSSION

The selectivity of ion separations in CE can be predicted from the tabulated values of the equivalent ionic conductivities [14], λ_i, which are directly related to the electrophoretic mobilities, μ_i, of the ions:

$$\mu_i = \lambda_i/F \tag{1}$$

where F is the Faraday constant. The electrophoretic mobilities, in turn, determine the velocity of the analyte under a given set of CE conditions. The resulting velocity of the ion is a sum of two contributions, the velocity of the ion and the velocity of the electroosmotic flow (EOF). The EOF is the bulk flow of liquid due to the effect of the electric field on the electrical double layer adjacent to the capillary wall. Hence it is the apparent mobility of the ions, which includes an electroosmotic component in addition to an electrophoretic component, that is measured in CE.

The velocity of migration in CE, v_{app}, is given in its simplest form by

$$v_{app} = (\mu_i + \mu_{EOF}) E \tag{2}$$

where μ_i is the electrophoretic mobility of the ion,

μ_{EOF} is the electroosmotic flow mobility and E is the electric field, or, in more detail, by [15]

$$v_{app} = \frac{L_d}{t} \cdot \frac{L_t}{V} \cdot E = \frac{L_d}{t} \tag{3}$$

where v_{app} is the measured migration velocity of the solute peak, E is the electric field strength, L_d is the length of the capillary from the injection end to the detector, L_t is the total length of the capillary, t is the measured migration time of the solute and V is the voltage across the capillary.

The velocity of the EOF is dependent on the charge of the capillary wall. The polarity of the charge on the wall determines the direction of the flow, while the amount of charge (zeta potential) determines the magnitude of the flow. For the anions, the differences in the equivalent ionic conductivities are sufficiently large that selective separations of large numbers of anions are possible [16] simply by modifying the velocity of the EOF. On the other hand, the equivalent ionic conductivities of the cations, and consequently their mobilities, are too close in many instances to expect selective separations based solely on the migration behavior of free cations. However, the apparent mobilities of cations may be affected by altering the charge on the analytes, by modifying the pH of the electrolyte or by the addition of complexing agents to the electrolyte.

An example of the effect of pH on the separation of inorganic cations is given by the separation of the alkali metals from the ammonium ion. In the separation of inorganic cations by CE, the positively charged ions migrate away from the positive power supply towards the detector, in the direction of the EOF. Smaller ions with higher equivalent ionic conductivities migrate the fastest (Fig. 1). In the separation in Fig. 2a, the cations elute in the order predicted by their equivalent ionic conductivities. As expected, potassium and ammonium co-elute at pH 6.15 owing to their identical equivalent ionic conductivities. By altering the pH of the electrolyte, the ionization of the alkali metal cations and their mobilities will be essentially unaffected. However, as the pH of the electrolyte is increased, the NH_4^+ becomes progressively less protonated (pK_b of $NH_4^+ = 4.75$) and its apparent mobility decreases. At pH 8.5, the apparent mobilities of K^+ and NH_4^+ become sufficiently different to permit an effective separa-
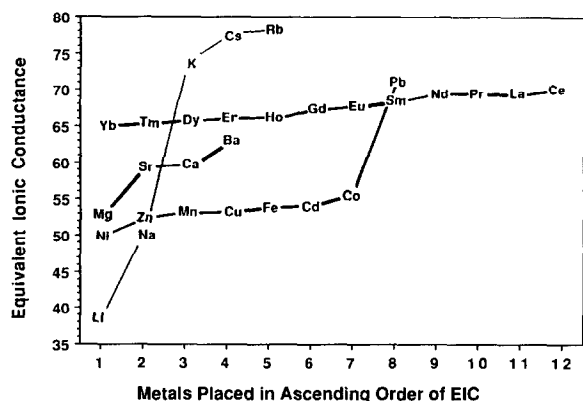
Fig. 1. Plot of equivalent ionic conductivities (EIC) of the Group IA, Group IIA, transition metal and lanthanide metal cations, placed in asending order of EIC. The closer the values, the more challenging the separation. It can be seen that the Group IA cations are more readily separated than the transition metals or the lanthanides.

tion (Fig. 2b). In addition to its effect on the mobility of the ammonium ion, the pH also affects the EOF. As the silanol groups on the capillary wall are weakly acidic (pK = 7–8) [17], the degree of their ionization is also dependent on the pH of the electrolyte. The higher the pH, the faster is the EOF. Thus, at high pH (*ca.* 8), in addition to the separation of K$^+$ from NH$_4^+$, the total analysis time also decreases (Fig. 2b).

The separation of the alkalil metals from the alkaline metals povides another example where poor resolution may be predicted from the equivalent ionic conductivities. As the equivalent ionic conductivities of strontium and calcium and also of sodium and magnesium are close, strontium and calcium co-migrate as do sodium and magnesium. In this instance, however, the addition of a weak complexing agent alters the apparent mobilities of the
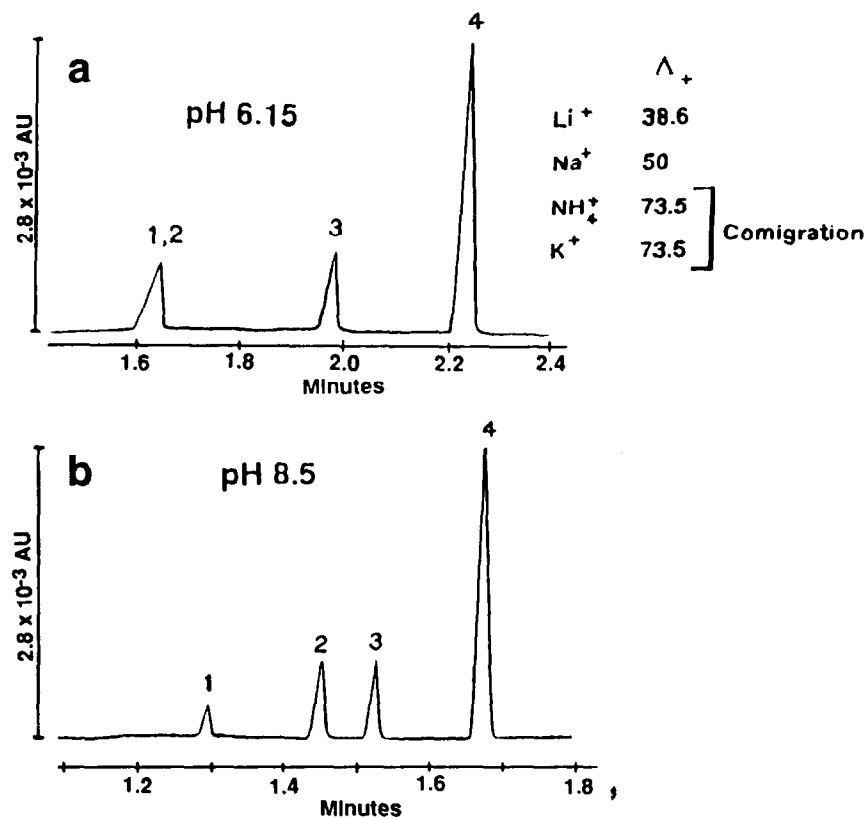

Fig. 2. (a) Separation of the Group IA metal cations in the presence of ammonia, using indirect photometric detection at 214 nm. Carrier electrolyte contains 5 mM morpholinoethanesulphonate adjusted to pH 6.15. A fused-silica capillary (75 μm I.D., 52 cm from the point of injection to the detector) was used for the separation and a positive voltage of 25 kV was employed. The sample was introduced into the capillary by hydrostatic injection, from a height of 10 cm, for 30 s. Potassium and ammonium have identical equivalent ionic conductivities and therefore co-elute. Peaks: 1 = potassium; 2 = ammonium; 3 = sodium; 4 = lithium. (b) CE separation of the Group IA metal cations at pH 8.5 in the presence of ammonia. CE conditions as in (a) except for pH. By altering the pH, the apparent mobilities of the analytes can be selectively modified. Peaks as in (a).
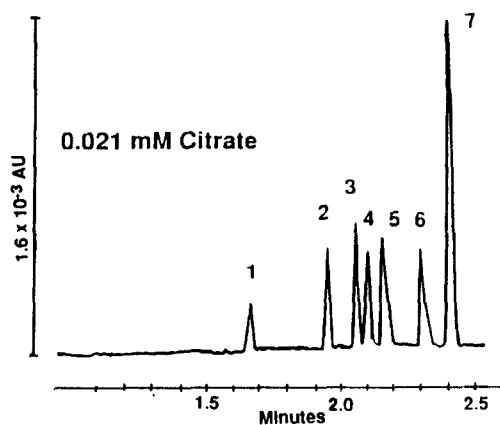
Fig. 3. Complete separation of Group IA and IIA metal cations due to the addition of a complexing agent (citrate) to the electrolyte. Carrier electrolyte, 5 mM Waters UVCat-1 (pH 5.5); capillary, 60 cm × 75 μm I.D. fused silica; voltage, 25 kV (positive); hydrostatic injection, 30 s from 10 cm height; indirect UV detection at 214 nm. Peaks: 1 = potassium; 2 = barium; 3 = strontium; 4 = sodium; 5 = calcium; 6 = magnesium; 7 = lithium.

cations sufficiently to permit a separation. An equilibrium, described by the stability constant K, is formed between the more mobile free cation and the slower, complexed form of the cation.

The interaction between the metal ion, M, and the complexing agent, CA, is described by the following equilibrium expression [13]:

$$K = \frac{[M(CA^-)_n]}{[M][CA^-]^n} \tag{4}$$

where n is the number of ligands. The apparent (observed) electrophoretic mobilities of the metal ions are then a combination of the mobilities of the free metal and the various complexes:

$$\mu_{app} = \alpha\,\mu_{M^-} + \beta\,\mu_{ML} + \ldots + \mu_{EOF}$$

where $\alpha + \beta = 1$ and are the mole fractions of each species in the capillary and μ_{M^-} and μ_{ML} are the mobilities of the free ion and a metal complex, respectively.

Although the equivalent ionic conductivities of the co-migrating cations are close, they are not identical. The addition of citrate at pH 5.5, a weakly complexing agent, is sufficient to effect a separation (Fig. 3). The additional benefit of the complexing agent is its effect on peak symmetry. The uncomplexed metals have a higher mobility than the co-ion in the background electrolyte and the peaks show various degrees of fronting [18,19]. The complexed cations posses lower apparent mobilities, which more closely match the mobility of the electrolyte. The peak shapes in Fig. 3, therefore, exhibit better peak symmetry.
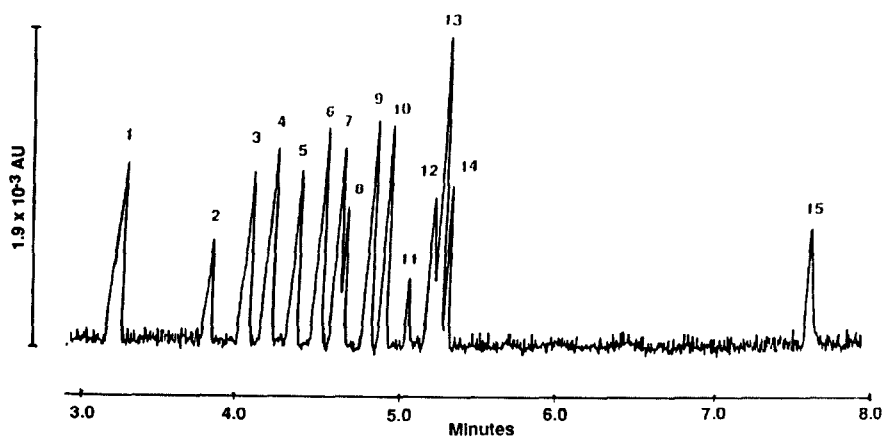

Fig. 4. Separation of alkali, alkaline earth and transition metal cations with the aid of an alternative complexing agent, HIBA. Carrier electrolyte, 5 mM Waters UVCat-1–6.5 mM HIBA (pH 4.4); capillary as described previously; voltage, 20 kV (positive); hydrostatic injection, as before; indirect UV detection at 214 nm. Peaks: 1 = potassium (0.8 ppm); 2 = barium (1.5 ppm); 3 = strontium (1.5 ppm); 4 = calcium (0.7 ppm); 5 = sodium (0.6 ppm); 6 = magnesium (0.4 ppm); 7 = manganese (0.8 ppm); 8 = cadmium (0.8 ppm); 9 = iron(II) (1.0 ppm); 10 = cobalt (0.8 ppm); 11 = lead (1.0 ppm); 12 = nickel (0.6 ppm); 13 = lithium (0.2 ppm); 14 = zinc (0.4 ppm); 15 = copper (0.6 ppm).
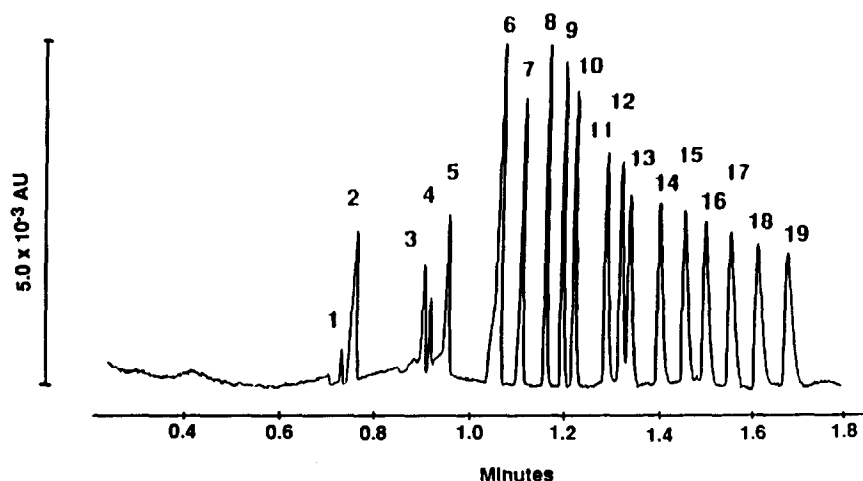
Fig. 5. Simultaneous separation of alkali, alkaline earth and lanthanide metal cations by CE. Carrier electrolyte, 10 mM Waters UVCat-1-4.0 mM HIBA (pH adjusted to 4.4 with acetic acid); capillary, 36.5 cm × 75 μm I.D. fused silica; voltage, 30 kV (positive); hydrostatic injection, 20 s from 10 cm height; indirect UV detection at 214 nm. Peaks: 1 = rubidium (2 ppm); 2 = potassium (5 ppm); 3 = calcium (2 ppm); 4 = sodium (1 ppm); 5 = magnesium (1 ppm); 6 = lithium (1 ppm); 7 = lanthanum (5 ppm); 8 = cerium (5 ppm); 9 = praseodymium (5 ppm); 10 = neodymium (5 ppm); 11 = samarium (5 ppm); 12 = europium (5 ppm); 13 = gadolinium (5 ppm); 14 = terbium (5 ppm); 15 = dysprosium (5 ppm); 16 = holmium (5 ppm); 17 = erbium (5 ppm); 18 = thulium (5 ppm); 19 = ytterbium (5 ppm).

In addition to citrate, HIBA has also been utilized as a complexing agent applicable to the separation of metals which have mobilities similar to one another [14]. HIBA is most commonly used in complexation reactions with the lanthanides [20], but was chosen here to aid in the separation of the alkali and alkaline earth metals from a group of transition metals (Fig. 4). The separations of 1 ppm

TABLE I

SENSITIVITIES FOR ALKALI, ALKALINE EARTH, TRANSITION AND LANTHANIDE METALS USING CAPILLARY ELECTROPHORESIS

Detection limits defined as 2 × noise in concentration units, using 10-cm hydrostatic injection and indirect UV detection at 214 nm for 30 s with the alkali, alkaline earth and transition metals and for 20 s with the lanthanide metals.

Cation	Sensitivity (ppb)[a]	Cation	Sensitivity (ppb)[a]	Cation	Sensitivity (ppb)[a]
Potassium	135	Manganese	120	Lanthanum	92
Barium	394	Cadmium	162	Cerium	77
Strontium	243	Iron	132	Praseodymium	81
Calcium	107	Cobalt	110	Neodymium	89
Sodium	96	Lead	388	Samarium	115
Magnesium	57	Nickel	118	Europium	120
Litium	18	Zinc	72	Gadolinium	138
		Copper	206	Terbium	146
				Dysprosium	154
				Holmium	163
				Erbium	170
				Thulium	183
				Ytterbium	197

[a] The American billion (10^9) is meant here.

TABLE II

REPRODUCIBILITY OF CE METHOD

Cation	R.S.D. (%) (n = 5)	
	Migration time	Peak area
Potassium	0.34	1.80
Sodium	0.37	2.60
Lithium	0.38	1.40
Barium	0.37	0.97
Strontium	0.38	1.07
Calcium	0.37	1.20
Magnesium	0.38	0.80

or less of each of fifteen cations was completed in 8 min with baseline resolution between the majority of the peaks. In ion chromatography, the separation of these fifteen cations requires two different separation modes and detection schemes [14].

The equivalent ionic conductivities of the lanthanides are even more similar to one another than those of the transition metals. The separation of the lanthanides from one another is therefore more chellenging than the separation of transition metals. Using HIBA as the complexing agent, a mixture containing less than 5 ppm of each of nineteen cations, including lanthanides and alkali and alka-
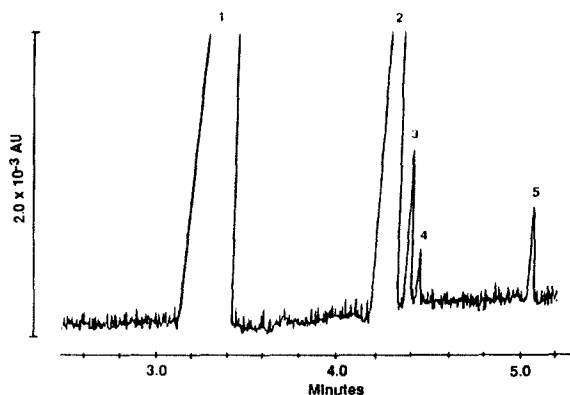

Fig. 6. CE analysis of a fermentation broth sample. Carrier electrolyte, 5 mM Waters UVCat-1–6.5 mM HIBA (pH 4.2), capillary, 60 cm × 75 μm I.D. fused silica; voltage, 20 kV (positive); hydrostatic injection, 30 s from 10 cm height; indirect UV detection at 214 nm. The original sample was diluted 1:100 before analysis. In the diluted sample, potassium and sodium are in the 100–1000 ppm range, while the other three analytes are in the 10–100 ppb range. Peaks: 1 = potassium; 2 = sodium; 3 = magnesium; 4 = manganese; 5 = zinc.

line earth metals, were separated in under 2 min (Fig. 5).

Minimum detectable injected concentrations for the metals, based on the peak height of twice the baseline noise, are given in Table I for the electrophoretic buffer containing UVCat-1 and HIBA at
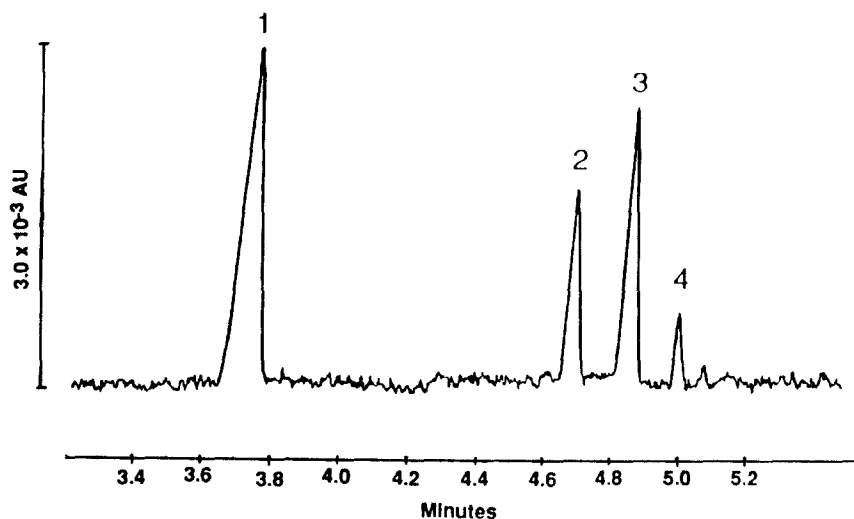

Fig. 7. CE analysis of a commercial cough syrup. CE conditions as in Fig. 6. The syrup was diluted 1:200 before analysis. Peaks: 1 = potassium; 2 = calcium; 3 = sodium; 4 = magnesium.

pH 4.4. The relative standard deviation (R.S.D.) in migration time for each of the Group IA and IIA cations, shown in Table II, was less than 1% ($n = 5$), and the R.S.D. in peak area was less than 2% (except Na = 2.6%). The linear dynamic range was almost two orders of magnitude above the detection limit ($R > 0.997$), the upper limit of calibration being due to loss of resolution rather than loss of linearity.

The potential of this technique for analyses for cations in complex matrices is shown in Figs. 6 and 7. With only a dilution required for the sample preparation, a fermentation broth and various cough medicines were both successfully analyzed for metal cations. In addition, this method shows great potential for use in environmental monitoring, to determine the concentrations of major cationic components in fresh, sea and waste waters rapidly, reproducibly and with high sensitivity.

REFERENCES

1 B. L. Karger, A. S. Cohen and A. Guttman, J. Chromatogr., 492 (1989) 585.
2 F. Foret and P. Boček, Electrophoresis, 11 (1990) 661.
3 W. G. Kuhr, Anal. Chem., 62 (1990) 403R.
4 F. E. P. Mikkers, F. M. Everaerts and T. M. P. Verheggen, J. Chromatogr., 169 (1979) 11.
5 F. Foret, M. Deml, V. Kahle and P. Boček, Electrophoresis, 7 (1986) 430.
6 L. Gross and E. S. Yeung, J. Chromatogr., 60 (1989) 169.
7 W. R. Jones and P. Jandik, Am. Lab., 22 (1990) 51.
8 S. Hjertén, Chromatogr. Rev., 9 (1967) 122.
9 T. Tsuda, K. Nomura and G. Nakagawa, J. Chromatogr., 264 (1983) 385.
10 X. Huang, T.-K. J. Pang, M. J. Gordon and R. N. Zare, Anal. Chem., 59 (1987) 2747.
11 J. L. Beckers, Th. P. E. M. Verheggen and F. M. Everaerts, J. Chromatogr., 452 (1988) 591.
12 F. Foret, S. Fanali, A. Nardi and P. Boček, Electrophoresis, 11 (1990) 780.
13 D. F. Swaile and M. J. Sepaniak, Anal. Chem., 63 (1991) 179.
14 P. R. Haddad and P. E. Jackson, Ion Chromatography (Journal of Chromatography Library, Vol. 46), Elsevier, Amsterdam, 1990.
15 P. D. Grossman, J. C. Colburn and H. H. Lauer, Anal. Biochem., 179 (1989) 28.
16 W. R. Jones, P. Jandik and A. Weston, J. Chromatogr., 546 (1991) 445.
17 M. L. Hair and W. Herte, J. Phys. Chem., 74 (1970) 91.
18 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, J. Chromatogr., 169 (1979) 1.
19 F. Foret, S. Fanali, L. Ossicini and P. Boček, J. Chromatogr., 470 (1989) 299.
20 T. Hirokawa, N. Aoki and Y. Kiso, J. Chromatogr., 312 (1984) 11.